PRESENCE AND LEVEL OF CAMPYLOBACTER SPP. ON BROILER CARCASSES THROUGHOUT THE PROCESSING PLANT

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Primary Audience: Plant Managers, Food Safety Officers, Researchers

SUMMARY

In order to create an effective HACCP program, it is important to know the effect of processing steps on microbial populations, including *Campylobacter*. To test for microbial populations, broiler carcasses were removed from the line in a commercial processing plant at six different sites. Care was taken to follow the same flock through the plant on each visit to eliminate the possibility of a flock effect masking any processing effect measured. On 6 different days, five birds were examined from each site for a total of 30 birds per site (six flocks). Sampling sites included: pre-scald, post-scald/pre-pick, post-pick, post-evisceration (immediately following removal of the viscera), pre-chill/post-final washer, and post-chill. All carcasses were sampled by whole carcass rinse and examined for total aerobes, coliform, generic *Escherichia coli* and *Campylobacter* populations. Results are reported as mean $\log_{10}$ CFU/mL of rinse fluid recovered. Overall bacterial populations decreased due to processing. However, coliform, *E. coli*, and *Campylobacter* counts, which were all depressed by scalding, increased after the birds moved through the picker.

Key words: Broiler, *Campylobacter*, *E. coli*, processing plant
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DESCRIPTION OF PROBLEM

Campylobacter (especially *C. jejuni*) are important human pathogens that cause foodborne illness ranging from self-limiting gastroenteritis to a number of severe sequela [1]. *Campylobacter* has been associated with poultry and poultry products and can be readily isolated from poultry production facilities [2, 3], birds entering the processing plant [4, 5], and the final product or fully processed broiler carcass [4, 6, 7]. In the early 1980s, research on the presence and level of *Campylobacter* in the poultry plant demonstrated that it could be isolated from water overflow samples and drip samples at various sites, which may represent areas of interest due to possible cross-contamination [4, 5].

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Campylobacter was also isolated from ceca, carcass swab, and neck skin samples during processing [4, 8]. Izat *et al.* [6] examined broiler carcasses by skin surface swabs at different stages of processing. This study, done before implementation of the Pathogen Reduction Act, showed that *Campylobacter* levels before scalding were about 1000 cells/1000 cm^2 of skin surface and fell through processing to 100–200 cells/1000 cm^2 of skin surface after chill. More recent research done in a small Scandinavian plant showed that vent cutter and eviscerator swab samples were positive, suggesting another possible cross-contamination site during processing [9].

Waldroup *et al.* [7] found that modifications of the poultry processing procedure can have a statistically significant effect on the levels of *Campylobacter* on the processed carcass. However, even with six process modifications, most carcasses (53–100%) in this 1992 study were still positive for *Campylobacter*.

Most work on *Campylobacter* in poultry plants was conducted prior to the enforcement of the Pathogen Reduction Act. This act has led to an increased number of wash/rinse procedures designed to reduce or eliminate fecal contamination of carcasses. Further, most of the reports make no reference to any effort to follow individual flocks through the plant. *Campylobacter*, like other pathogens, is somewhat flock-related in that most flocks test positive at the end of grow-out but some do not [2].

The present study was undertaken to examine the current levels of *Campylobacter* on broilers by following birds from six different flocks as they moved through the processing plant. Such within-flock monitoring should show the effect of each processing step and not the level of flock contamination. A secondary objective was to compare the presence and level of *Campylobacter* to the total aerobic bacterial, coliform, and generic *E. coli* populations on the same birds. Such comparisons are important because these populations are often used as an indication of microbiological quality of the processed broiler. This information may be useful to commercial processors in their effort to design an effective HACCP program for control of human pathogens on poultry and poultry products.

Materials and Methods

SAMPLING SCHEME

Samples were collected at a commercial broiler processing plant in north Georgia. Carcasses were collected at six sites within the plant: A) immediately prior to scald (after bleed-out), B) post-scald prior to entering the picker, C) post-pick before transfer to the evisceration line, D) immediately following the removal of the viscera (prior to the inside-outside washer station), E) immediately prior to entering the pre-chill tank (after the final washer), and F) post-chill before re-hang. Samples were taken at each site sequentially through the plant such that the same truckload of birds was sampled through the plant on each sample day in order to eliminate confounding flock effects. At each sample site, five carcasses were removed from the line, using new clean latex gloves for each. Carcasses were placed individually into sterile plastic bags that were sealed and covered with ice. After collection of all carcasses, the samples were transported to the lab and held on ice until analyzed (within 2 hr of collection). Six trips were made to the plant; six flocks were sampled and the total sample number was 30 carcasses for each sample site.

PLANT SPECIFICATIONS

All birds were taken off feed 6–8 hr prior to leaving the farm, and were transported in standard transport cages. Holding time at the plant ranged from 2–4 hr. Birds were dumped from the transport cages, hung on the kill line, shackled, and stunned with a brine stunner (12–20 volts pulsating DC). Birds were killed by severing the carotid artery and the jugular vein on one side of the neck, and were bled for 75 sec prior to entering the scald tank.

The scalder was a three-stage counterflow with 30–50 ppm Cl added to startup and makeup water. The carcasses were in the scalder for 2.5 min at an average temperature of 55.4°C. After leaving the scalder the carcasses passed through five banks of pickers, each with 30–50 ppm Cl spray. After picking, carcasses passed through a final picking room spray with 30–50 ppm Cl.

The carcasses progressed through an automatic re-hang and were eviscerated and inspected. After inspection, all carcasses were subjected to an inside-outside wash, and a high flow, high pressure washer. Both washers and all evisceration spray nozzles used water with 30–50 ppm Cl. Carcasses underwent a pre-chill at 12–14°C for 10–12 min and a final chill treatment at 2–4°C for 50–55 min. Both chill tanks were treated with 20–40 ppm Cl.

SAMPLE METHOD

Sterile distilled water was added to each sample within the sterile bag. Carcasses from sites A and B (feathered carcasses with feet intact) received 300 mL of water; birds from sites C, D, E, and F each received 100 mL of water [10]. Each carcass was placed in a bucket attached to a mechanical shaker and rinsed thus for 1 min. [11]. Each carcass was aseptically removed from the bag, allowed to drain briefly into the bag, then discarded. The rinse liquid was cultured for *Campylobacter*, total aerobic bacterial, coliform, and *E. coli* populations.

CULTURE METHODS

Serial dilutions were made in phosphate buffered saline, and *Campylobacter* was enumerated by plating in duplicate onto the surface of Campy-cefex agar [12]. Plating was achieved by spreading 0.1 mL on the surface of each plate with a sterile plastic inoculating loop. Plates were then incubated at 42°C for 36 hr in a microaerophilic environment (5% O_2, 10% CO_2, and balance N_2). CFUs characteristic of *Campylobacter* were counted. Each colony type counted as *Campylobacter* from each sample was confirmed as a member of the genus by examination of cellular morphology and motility on a wet mount under phase contrast microscopy. Each colony type was further characterized as a member of the species *jejuni*, *coli*, or *lari* by a positive reaction on a latex agglutination test kit [13]. Total aerobic bacterial populations were enumerated on plate count agar [14] by plating 0.1 mL from a serial dilution of the rinse diluent in duplicate on the surface of the agar; this was spread and incubated at 37°C for 18–24 hr prior to counting the resulting CFUs. Coliform and *E. coli* counts were made by plating 1 mL from a serial dilution of the rinse diluent onto duplicate *E. coli* Petrifilm plates [15]. Petrifilm plates were incubated at 37°C for 18–24 hr and colony types characteristic of coliforms and *E. coli* were counted.

STATISTICAL ANALYSES

Duplicate counts were averaged and these numbers were transformed by $\log_{10}$. The $\log_{10}$ values were used to make geometric means within each site, or flock. These means were compared by student's *t*-test. Pearson correlations were performed to study the relationships between different bacterial populations measured. All statistical manipulations were performed with Statistica release 5 [16].

RESULTS AND DISCUSSION

The mean bacterial populations detected on whole carcass rinse samples from the six in-plant sample sites are reported in Table 1. The numbers were highest for all populations measured (total aerobes, coliform, *E. coli*, and *Campylobacter*) when the carcasses were sampled pre-scald. All counts dropped significantly (P < .05) after the carcasses were scalded. After defeathering, the counts of

TABLE 1. Mean $\log_{10}$ CFU/mL of rinse from whole carcass rinse samples collected at various sites in the processing plant (n = 30)

SITE	TOTAL AEROBIC	COLIFORM^A	E. COLI^A	CAMPYLOBACTER^A
Pre-scald	$6.8^a \pm 0.1^B$	$5.0^a \pm 0.2$ (30)	$4.3^a \pm 0.2$ (28)	$4.7^a \pm 0.5$ (25)
Post-scald	$5.0^b \pm 0.2$	$2.9^b \pm 0.2$ (30)	$2.1^b \pm 0.3$ (30)	$1.8^b \pm 0.3$ (19)
Post-pick	$5.0^b \pm 0.1$	$3.4^c \pm 0.3$ (28)	$2.8^c \pm 0.2$ (26)	$3.7^c \pm 0.3$ (24)
Post-evisceration	$4.5^c \pm 0.1$	$3.1^b \pm 0.1$ (30)	$2.2^b \pm 0.2$ (30)	$3.4^c \pm 0.3$ (26)
Pre-chill	$3.6^d \pm 0.2$	$2.2^d \pm 0.1$ (30)	$1.5^d \pm 0.2$ (30)	$2.3^d \pm 0.3$ (25)
Post-chill	$2.9^e \pm 0.3$	$1.9^d \pm 0.2$ (30)	$1.1^e \pm 0.4$ (30)	$1.5^{bd} \pm 0.4$ (22)

^{A}These counts are mean values from only the samples found positive within the limits of detection. Numbers in parentheses are the number of samples positive out of 30 tested.

^{B}±95% confidence interval.

$^{a\text{–}e}$Figures within a column without like superscripts are significantly different by student's t-test (P < .05).

coliforms, *E. coli*, and *Campylobacter* had increased significantly ($P<.05$), while total counts did not change. *Campylobacter* incidence and population increased between the end of scald and the end of the defeathering procedure. Increase in *Campylobacter* counts following defeathering has been previously reported [6, 8]. It has been suggested that the rubber fingers in the mechanical picker act to cross-contaminate birds that previously had low or undetectable levels of *Campylobacter* [8, 17]. Positive samples of feather picker drip water tend to support this hypothesis [4]. Another possibility is that contact between the picker fingers and the abdomen of the carcass causes release of gut contents still present in the lower bowel, leading to an increase in the populations of microorganisms commonly found in gut contents, such as coliforms and *Campylobacter*. As the carcasses moved through the rest of the processing plant, all counts decreased. Carcasses examined after immersion chilling had the lowest mean counts of all populations measured.

Each post-pick processing step (evisceration, final wash, chill) resulted in a significant decrease in total aerobic bacterial counts and *E. coli* numbers recovered. However, coliform counts on carcasses sampled post-chill were not significantly lower ($P<.05$) than those taken immediately prior to chill. Overall, *Campylobacter* counts dropped as the flocks moved through the plant. However, neither the decrease from post-pick to post-evisceration nor from post-final wash (pre-chill) to post-chill were significant. Interestingly, *Campylobacter* counts detected on positive carcasses leaving the chill tank were not significantly different from those detected on positive carcasses leaving the scald tank. When the carcasses exited the chill tank, the *Campylobacter* incidence and population were once again roughly the same as those recorded post-scald.

The variation in *Campylobacter* population by flock is shown in Table 2. The data from the pre-scald sample site highlight the variation possible in the presence and numbers of *Campylobacter* on birds at entry to the plant. In Flock 1, only two of five carcasses were positive pre-scald with a mean population of 2.9 $\log_{10}$ CFU/mL rinse, whereas in Flock 5 five of five birds were positive at levels of 5.8 $\log_{10}$ CFU/mL rinse. In general, less variation is seen in the level of *Campylobacter* on carcasses at removal from the chill tank. With most flocks, post-chill *Campylobacter* levels were found to be near or at our limit of detection (10 cells/mL rinse). However, carcasses from Flock 5 had higher counts of *Campylobacter* coming out of the chill tank than going into the tank. Since post-chill is the only site where Flock 5 varied appreciably from other flocks, it seems likely that this might be due to some difference in processing parameters on that day rather than a flock effect. Aside from the post-chill outlier seen in Flock 5, all the flocks had the same type of *Campylobacter* count response at each processing step with respect to both direction and relative difference.

A significant ($P<.01$) correlation was found between *Campylobacter* counts and total counts at the post-chill sample site. The R^2 for this correlation was 0.73, indicating that more than 70% of the variation seen in total aerobic bacterial numbers may be explained by a relationship to the *Campylobacter*

TABLE 2. Mean $\log_{10}$ Campylobacter CFU/mL of rinse on positive chicken carcasses from various sites in the plants as affected by flock ($n \leq 5$)

SITE	FLOCK[A]					
	1	2	3	4	5	6
Pre-scald	2.9 (2)	5.0 (5)	5.0 (5)	3.1 (3)	5.8 (5)	4.6 (5)
Post-scald	1.0 (1)	2.0 (3)	1.7 (5)	2.4 (1)	2.4 (4)	1.5 (5)
Post-pick	<3.0 (0)	3.2 (5)	4.5 (5)	3.1 (4)	4.1 (5)	3.7 (5)
Post-evisceration	1.6 (1)	3.2 (5)	3.7 (5)	2.53 (5)	4.0 (5)	3.7 (5)
Pre-chill	<1.0 (0)	2.1 (5)	3.3 (5)	2.0 (5)	1.6(5)	2.7 (5)
Post-chill	0.9 (4)	1.2 (2)	1.1 (3)	0.9 (4)	3.2 (5)	1.1 (4)

[A]Numbers in parentheses are the number of samples found positive within the limits of detection out of a total of five tested.

population. However, this relationship requires more investigation, because it is possible that both the *Campylobacter* and total counts are related to some other unmeasured factor. Statistically significant correlations were also noted between *Campylobacter* counts and coliforms at the post-scald and post-chill sites, and between *Campylobacter* and *E. coli* at the post-chill site. However, although the P values were low ($P < .01$), the R^2 values were also low, indicating that only a small percentage (29–30%) of the variation in *Campylobacter* counts could be attributed to the relationship to coliform or *E. coli* counts. Therefore, it would seem neither *E. coli* nor coliform counts are useful in predicting *Campylobacter* populations on broiler carcasses.

CONCLUSIONS AND APPLICATIONS

1. Overall, commercial poultry processing lowers the populations of *Campylobacter* found on broiler carcasses.
2. *Campylobacter* populations and the percentage of birds positive decline after scalding but increase significantly during the picking process.
3. Each post-pick processing step tends to lower the level of *Campylobacter* on carcasses.
4. The incidence and level of *Campylobacter* contamination can differ noticeably between flocks.
5. *Campylobacter* populations on positive birds exiting the chill tank cannot be predicted by *E. coli* or coliform populations.

REFERENCES AND NOTES

1. **Nachamkin, I.**, 1997. Campylobacter jejuni. Pages 159–170 in: Food Microbiology Fundamentals and Frontiers. M.P. Doyle, L.R. Beuchat, and T.J. Montville, eds. ASM Press, Washington, DC.

2. **Stern, N.J., M.R. Clavero, J.S. Bailey, N.A. Cox, and M.C. Robach**, 1995. Campylobacter spp. in broilers on the farm and after transport. Poultry Sci. 74:937–941.

3. **Stern, N.J. and M.R. Robach**, 1995. Non-destructive sampling of live broilers for Campylobacter. J. Appl. Poultry Res. 4:182–185.

4. **Wempe, J.M., C. Genigeorgis, T.B. Farver, and H.I. Yusufu**, 1983. Prevalence of Campylobacter jejuni in two California chicken processing plants. Appl. Environ. Microbiol. 45:355–359.

5. **Yusufu, H.I., C. Genigeorgis, T.B. Farver, and J.M. Wempe**, 1983. Prevalence of Campylobacter jejuni at different sampling sites in two California turkey processing plants. J. Food Prot. 46:868–872.

6. **Izat, A.L., F.A. Gardner, J.H. Denton, and F.A. Golan**, 1988. Incidence and level of Campylobacter jejuni in broiler processing. Poultry Sci. 67:1568–1572.

7. **Waldroup, A., B.M. Rathgeber, R.H. Forsythe, and L. Smoot**, 1992. Effects of six modifications on the incidence and levels of spoilage and pathogenic organisms on commercially processed postchill broilers. J. Appl. Poultry Res. 1:226–234.

8. **Acuff, G.R., C. Vanderzant, M.O. Hanna, J.G. Ehlers, F.A. Golan, and F.A. Gardner**, 1986. Prevalence of Campylobacter jejuni in turkey carcass processing and further processing of turkey products. J. Food Prot. 49:712–717.

9. **Berndtson, E., M.L. Danielsson-Tham, and A. Engvall**, 1996. Campylobacter incidence on a chicken farm and the spread of Campylobacter during the slaughter process. Intl. J. Food Microbiol. 32:35–47.

10. **Cox, N.A., J.E. Thomson, and J.S. Bailey**, 1981. Sampling of broiler carcasses for Salmonella with low volume water rinse. Poultry Sci. 60:768–770.

11. **Dickens, J.A., N.A. Cox, J.S. Bailey, and J.E. Thomson**, 1985. Automated microbiological sampling of broiler carcasses. Poultry Sci. 64:1116–1120.

12. **Stern, N.J., K. Wojton, and B. Kwiatek**, 1992. A differential selective medium and dry ice generated atmosphere for recovery of Campylobacter jejuni. J. Food Prot. 55:514–517.

13. Integrated Diagnostics Inc., Baltimore, MD 21227.

14. Difco, Detroit, MI 48232.

15. 3M Health Care, St. Paul, MN 55144.

16. Statsoft, Tulsa, OK 74104.

17. **Stern, N.J., C.E. Lyon, and M.T. Musgrove**, 1995. Bacterial quality of broilers and alternative processing procedures. J. Appl. Poultry Res. 4:164–169.

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